INDUCTION OF PUPAL DIAPAUSE IN *PAPILIO AEGEUS AEGEUS* DONOVAN AND *GRAPHIUM SARPEDON CHOREDON* (C. & R. FELDER) (LEPIDOPTERA: PAPILIONIDAE)

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Abstract

Data are presented on the induction of pupal diapause in the papilionid butterflies, *Papilio aegeus aegeus* Donovan and *Graphium sarpedon choredon* (C. & R. Felder). Laboratory studies showed diapause in both species to be facultative and induced primarily by short larval photoperiods. Temperature had a modifying influence on the incidence of diapause, particularly in *P. a. aegeus* which showed continuous development at warm temperatures. The diapause response of *G. s. choredon* showed greater coupling to photoperiod at warm temperatures, but the non-diapausing effect of long photophases was negated by cool temperatures. Short photophases at 25°C resulted in hastened larval development and reduced pupal weights in both species.

Introduction

A number of studies have been reported concerning the overwintering strategies of various species of swallowtail butterflies (Papilionidae) in temperate areas of North America and Japan. Most have been shown to exhibit a facultative pupal diapause as a result of environmental stimuli (usually photoperiod and/or temperature) operating during the larval stage (Oliver 1969; Ichinose 1974; Sims and Shapiro 1983; Shimada 1983; Endo and Murakami 1985). No detailed studies on the overwintering biology of Australian swallowtail butterflies have been reported, although at least 5 species occur in the temperate zone (Common and Waterhouse 1981).

Both *Papilio aegeus aegeus* Donovan and *Graphium sarpedon choredon* (C. & R. Felder), are familiar butterflies over much of eastern Australia. *P. a. aegeus* is widely distributed but is most common in coastal areas of Queensland and New South Wales. It is occasionally considered to be a minor pest of young citrus (Hely *et al*, 1982). *G. s. choredon* although common, is confined to the coastal strip from Cape York to a little south of Sydney. Both species occur in tropical and temperate areas with probable year-round production of generations in the former and a winter period of inactivity in the latter. Overwintering in both species is known to occur in the pupal state (Common and Waterhouse 1981). However, apart from a statement by these authors that larvae of *P. a. aegeus* produce diapausing pupae if exposed to a daylength of less than 14 hours, no information is available on the induction or nature of winter dormancy in *P. a. aegeus* or *G. s. choredon*.

This paper presents the results of experiments designed to investigate the effect of temperature and photoperiod on the induction of pupal diapause in *P. a. aegeus* and *G. s. choredon*.

Materials and Methods

Butterflies used in this study were obtained from gravid females (*P. a. aegeus*) or eggs (*G. s. choredon*) collected in the Sydney area during 1985/86. Females of *P. a. aegeus* oviposited on potted citrus (*Citrus* spp., Rutaceae) in a wooden framed, muslin covered cage maintained at 28°C under constant illumination. Eggs of *G. s. choredon* were readily found on terminal shoots of *Cinnamomum camphora* (L.) (Lauraceae) and transported to the laboratory. Rearing of larvae of both species was carried out in perspex cylinders (30 x 15 cm) with muslin lids, using potted citrus or cut *C. camphora* in water as food for *P. a. aegeus* and *G. s. choredon* respectively.

Induction of pupal diapause was studied at 3 temperatures (20°, 25° and 30°C) and 2 photoperiods (LD 10:14, 15:9). These conditions were provided by environmental chambers with temperature variance of ±1°C and time-controlled fluorescent lighting. Eggs were placed in the chambers before observable embryonic development occurred. Upon completion of larval development, pupae were removed and stored at 30°C, LD 15:9. Pupae were judged to be in diapause if eclosion had not occurred within 24 days under these conditions. Mean number of days from pupation to eclosion for non-diapausing *P. a. aegeus* and *G. s. choredon* under this temperature/photoperiod regime was 11.9 ± 0.4, range 11-13, n = 15 and 12.8 ± 0.7, range 12-14, n = 12, respectively. In some experiments data were collected on duration of larval period and pupal weights. In an additional experiment, larvae of both species were reared outdoors under natural conditions of temperature and photoperiod during late summer and autumn 1986.

Results

The effect of temperature and photoperiod on the induction of pupal diapause in *P. a. aegeus* and *G. s. choredon* in the laboratory is shown in Table 1. Both species exhibited a facultative pupal diapause induced by short day photoperiods during larval development. In *P. a. aegeus* the diapause response to short days was overridden by warm temperatures. Thus at 25°C, 33% of pupae produced under short day conditions were non-diapause and at 30°C short-day-induced diapause was prevented. However, in *G. s. choredon* a temperature of 25°C failed to override the diapause-producing effect of short photoperiod, whilst only a small percentage of individuals failed to diapause at 30°C under LD 10:14. In this species, cool (20°C) temperatures negated the effect of long day photoperiods and produced a high incidence of diapause. 100% incidence of diapause was recorded only for *G. s. choredon* at 20° and 25°C under short photoperiod. In *P. a. aegeus* some non-diapausing pupae were produced even under cool temperatures and short daylength.

Short day conditions for rearing at 25°C resulted in individuals of both species spending a significantly shorter time as larvae than under long day conditions (Table 2). There was also a corresponding reduction in pupal weights of the faster developing larvae. A similar developmental response

Table 1. Effect of temperature and photoperiod on induction of pupal diapause in *P. a. aegeus* and *G. s. choredon.*

Rearing temperature (°C)	Photoperiod (L:D)	n	% Diapause
P. a. aegeus			
20	10 : 14	12	92
20	15 : 9	12	0
25	10 : 14	12	67
25	15 : 9	12	0
30	10 : 14	12	0
30	15 : 9	12	0
G. s. choredon			
20	10 : 14	12	100.0
20	15 : 9	14	71.4
25	10 : 14	12	100.0
25	15 : 9	12	0
30	10 : 14	12	91.6
30	15 : 9	12	0

Table 2. Larval durations and pupal weights of *P. a. aegeus* and *G. s. choredon* reared under different conditions of temperature and photoperiod.

Temperature photoperiod	n	Mean larval duration (days $\pm$ SE)	Mean pupal wt. (mg $\pm$ SE)
P. a. aegeus			
25°C			
L:D 15 : 9	6	27.1 $\pm$ 1.1	1467.3 $\pm$ 182.0
L:D 10 : 14	6	24.2 $\pm$ 1.9*	1162.9 $\pm$ 97.9
G. s. choredon			
20°C			
L:D 15 : 9	8	38.0 $\pm$ 3.9	831.9 $\pm$ 146.5
L:D 10 : 14	8	26.0 $\pm$ 2.4**	873 $\pm$ 124.4
25°C			
L:D 15 : 9	8	26.4 $\pm$ 1.9	1172.6 $\pm$ 86.0
L:D 10 : 14	9	19.1 $\pm$ 1.**	906.6 $\pm$ 183.2
30°C			
L:D 15 : 9	5	13.0 $\pm$ 2.4	978.4 $\pm$ 79.0
L:D 10 : 14	8	14.1 $\pm$ 2.4	995.9 $\pm$ 42.7

*$P < 0.01$ **$P < 0.001$ Significantly shorter duration or lighter weight

to photoperiod was also noted for *G. s. choredon* larvae reared at 20°C although pupal weights did not differ significantly. No photoperiodically-induced differences in developmental duration and pupal weights were detected at 30°C.

Larvae of both species reared under natural conditions of temperature and photoperiod during late January-late March produced diapausing pupae (Table 3). During this time daylengths declined from 14-12 h and mean daily maximum and minimum temperatures ranged from 23-27°C and 15-19°C, respectively.

Discussion

The results presented indicate that *P. a. aegeus* and *G. s. choredon* undergo a facultative pupal diapause in winter induced primarily by short photoperiods operating during the larval stage. The diapause-inducing effect of short daylengths is modified by temperature, particularly in *P. a. aegeus*. Total avoidance of diapause is possible in this species at warm temperatures. However, in *G. s. choredon* warm temperatures only allow a small proportion of individuals to continue direct development under short photoperiods.

Diapause appears to be fixed more strongly as an overwintering strategy in *G. s. choredon* than *P. a. aegeus*. Under summer temperatures of 20-25°C, short days produced 100% diapause response amongst larvae of *G. s. choredon*. A high incidence of diapause was also obtained under long daylengths at 20°C. Conversely, the diapause response of *P. a. aegeus* shows greater flexibility with 100% diapause not achieved under any temperature/photoperiod condition tested. This species has the capacity, if temperatures are high enough, to continue development despite a short photoperiod. Larvae of both species reared outdoors under natural late summer-autumn conditions in Sydney, produced only diapausing pupae. It is difficult to compare the results obtained in the laboratory, under fixed temperatures and photoperiods, with data obtained under varying ambient conditions. However, the effective mean temperature experienced by the outdoor larvae was probably close to 20°C in most instances, and the average mid-larval stage photophase about 13 h.

The critical photoperiod for diapause induction in *P. a. aegeus* and *G. s. choredon* has not been determined accurately. However, the data for *P. a. aegeus* suggest that at 25°C the critical photoperiod (inducing diapause in 50% of individuals) is little more than 10 h. The substantial modifying effect of temperature on diapause induction in this species indicates that the critical photoperiod is dependent on temperature. This is less likely to be the case with *G. s. choredon*, with limited data indicating a critical photoperiod of about 13 h at 30°C (James, unpublished observations). The precise photo-sensitive stage for diapause induction during larval

development is unknown for either species but is likely to be during mid-larval life as it is in a number of other papilionids (Endo *et al.* 1985; Shimada 1985).

An interesting observation was the hastening of larval development in both species under short photoperiod at 25°C. This may be an adaptation to ensure complete larval development before the deterioration of environmental conditions, signalled by short photoperiod. The resulting spring generation of butterflies may also be better adapted for dispersal, by virtue of their smaller size and probable lower wing-loading ratio (Angelo and Slansky 1984). Development under cool temperatures and short photoperiods has been shown to produce "migration-adapted" adults in two Australian vanessid butterflies (James 1987).

The difference in diapause induction response between *P. a. aegeus* and *G. s. choredon* may reflect their different geographical origins. The suppression of photoperiodic reaction by high temperatures in *P. a. aegeus* may be an adaptation to continuous development under short days in the tropics. Conversely, strict photoperiodic control of diapause in *G. s. choredon* may indicate evolution of this species in more temperate regions. The flexibility of diapause response in *P. a. aegeus* should result in continuous development in warm areas where winter breeding of *G. s. choredon* would be constrained by short photoperiods Adults of *P. a. aegeus* may be taken throughout the year in most of Queensland. The seasonal breeding status of *G. s. choredon* in northeastern Australia is less certain (Common and Waterhouse 1981). A number of sub-species of both *P. a. aegeus* and *G. s. choredon* occur in the Australian and Oriental zoogeographical regions. Comparative studies on the diapause response of these races would yield interesting biological as well as evolutionary information, concerning the spread of these species in the Pacific region.

Table 3. Induction of pupal diapause in *P. a. aegeus* and *G. s. choredon* under natural conditions of temperature and photoperiod.

	n	Photoperiod (h)	Temperature °C Max $\bar{x} \pm SE$	range	Min $\bar{x} \pm SE$	range	% Diapause
P. a. aegeus							
27.1 - 5.III	12	13.9 - 12.5	24.1 ± 3.8	20 - 28	19.1 ± 2.9	16 - 22	100
16.II - 24.III	6	13.4 - 12.1	23.2 ± 3.4	16 - 31	15.6 ± 2.5	9 - 20	100
G. s. choredon							
18.I - 22.II	6	14.0 - 13.0	26.6 ± 4.1	20 - 32	17.5 ± 3.0	12 - 21	100
3.II - 10.III	6	13.5 - 12.5	25.2 ± 3.9	20 - 29	15.4 ± 2.9	12 - 19	100
23.II - 28.III	6	13.1 - 12.0	22.9 ± 3.6	16 - 31	15.5 ± 2.7	9 - 20	100

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